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Environmental effects on wing shape and wing size of *Argia sedula* (Odonata: Coenagrionidae)

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Well-adapted flight morphology must allow for efficient behavioral activities. Wing shape has been shown in a variety of species to be influenced by environmental conditions. Analysis of wing shape using geometric morphometrics provides a visualization of wing shape variations. This study examined the effects of varied environments on wing shape and wing size of the damselfly *Argia sedula* in central Texas. Comparisons were made (1) between populations collected early in the flight season versus those collected late in the flight season; (2) between populations collected at different locations, and (3) among populations collected from the same locations during several annual flight seasons. We found widespread differences in both wing shape and body size in males and females among most environments examined. Male and female damselflies collected early varied significantly from those collected late in the flight season for all locations and years sampled. Damselflies emerging early in the flight season were significantly larger than those emerging late in the season. Significant differences in wing shape and size occurred in comparisons of male and female damselflies collected in different years at the same location. In comparing damselflies collected at different locations, neither females nor males varied significantly in wing shape. Size varied in only one male comparison between locations. Our results suggest that differences in seasonal and year-to-year environmental conditions, but not geographical location, frequently influence wing shape and wing size in *A. sedula*, and quite possibly in other damselfly species.

Keywords: annual; damselfly; dragonfly; flight; geometric morphometrics; locality; seasonal; wings

Introduction

Wing shape greatly influences flight performance, energetic costs, and maneuverability of flight (Gyulavári et al., 2017; Lee & Lin, 2012; Outomuro, Rodríguez-Martínez, Karlsson, & Johansson, 2014), all of which in turn affect fitness (Breuker, Gibbs, Van Dongen, Merckx, & Van Dyck, 2010; Outomuro, Adams, & Johansson, 2013). Behavioral activities such as avoiding predators, foraging, defending territories, mating, and locating oviposition sites depend on efficient flight (Breuker et al., 2010; Westfall & May, 2006). Thus, flight morphology in winged insects is likely under strong selection to optimize these activities specifically involving staying aloft, maneuverability, and thrust (Betts & Wootten, 1988; Dellicour et al., 2017). All of these actions depend on the shapes of individuals' bodies and wings (Sadeghi & Dumont, 2014).

Variations in morphologies result from developmental and environmental processes producing differences among individuals and populations (Hoffman, Collins, & Woods, 2002). Environmental conditions, as well as genetic make-up, affect and constrain these differences (Outomuro

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& Johansson, 2011). Selection may operate on plastic traits such as flight morphology necessary for survival and reproduction (Breuker et al., 2010). Wing shape results from the combined and sometimes conflicting action of natural and sexual selection pressures (Outomuro, Bokma, & Johansson, 2012) and from structural and aerodynamic constraints (Outomuro et al., 2013).

Developmental conditions dictate much of the morphological variation in the adult stage. For example, adult body size and shape depend on nutrients available to larvae as well as local environmental conditions including temperature and water quality (Corbet, 1999; Lee & Lin, 2012). Organisms that inhabit different environments (aquatic and terrestrial) as larvae and adults must optimize responses to selection pressures operating in each environment (Bybee et al., 2016). Notably, populations of the same damselfly species can live and develop in a considerable range of habitats with quite varied biotic and abiotic factors (Gibbs, Wiklund, & Van Dyck, 2010). For example, developmental responses to local factors and geographic variation contributed to significant differences in wing shape among Eurasian populations of the damselfly, *Calopteryx splendens* (Sadeghi & Dumont, 2014). Differences in subtle natural selective pressures among habitats can cause observable variations in wing shape (Outomuro & Johansson, 2011). Plasticity of wing development is advantageous for species occupation of varied environments and development of optimal flight design for local conditions (Breuker et al., 2010: Gibbs et al., 2010).

Environmental conditions vary in time, e.g. season, as well as in space, e.g. habitat conditions. Varied seasonal conditions should promote varied optima for flight morphologies (Van Dyck & Wiklund, 2002). Variation in flight morphology through the flight season is expected since (1) constraints during development often influence body size and wing morphology (Gibbs et al., 2010; Van Dyck & Wiklund, 2002); and (2) flight morphology is plastic in response to environmental conditions such as developmental temperature (Bots et al., 2009; Westfall & May, 2006). For example, developmental temperatures influenced complex changes in *Drosophila* body shape with low temperatures resulting in longer wings (Pétavy, Morin, Moreteau, & David, 1997). Cooler seasonal temperatures allow longer flight in the butterfly *Pararge aegeria* (Gibbs et al., 2010). Each seasonal cohort of *P. aegeria* experienced different local weather conditions during larval development, and responded with within-year variation in adult phenotypes (Van Dyck & Wiklund, 2002).

Habitat conditions change annually as well as on a more or less predictable seasonal basis. Life cycle stages influenced by environmental changes may initiate morphological adaptations (Gallesi, Mobili, Cigognini, Hardersen, & Sacchi, 2016). For example, the variable length of larval life in damselflies depends largely on temperature and photoperiod (Westfall & May, 2006). This temporal environmental heterogeneity supports flexible development to produce the most adaptive phenotypes possible within environmental constraints. For example, butterfly morphologies are highly sensitive to weather and climate, particularly rainfall levels (Gibbs et al., 2010). Changes in wing-aspect ratio of *Drosophila melanogaster* have been related to environmental conditions, especially temperature extremes (Hoffman et al., 2005). Selection may promote plasticity of flight morphology and ensure that the development of these traits is flexible enough for rapid responses to varying conditions across space and time (Breuker et al., 2010).

The purpose of this study is to examine the sensitivity of damselfly developmental plasticity to variations in location, seasonality, and year-to-year habitat conditions. We use landmark-based geometric morphometrics to quantify and analyze wing shape in the damselfly *Argia sedula* (Hagen, 1861). Wing shape, a multivariate trait, can be examined using Procrustes techniques in geometric morphometrics, which removes variation in orientation, position and scale but preserves shape (Bots et al., 2012; Hoffman et al., 2002; Ren, Bai, Yang, Zhang & Ge, 2017). Correction for the effects of allometry was accomplished using the residuals from a regression of shape on centroid size in subsequent analyses (Klingenberg, 2010). We searched for the effects of

habitat, seasonality, and annual variation on wing shape and wing size of this damselfly. Specifically, our comparisons included differences in wing shape and wing size for (1) adults developing and emerging from three habitat types, river, stream, and wetlands; (2) adults emerging early in the flight season versus those emerging late in the flight season; and (3) adults emerging in different years from the same habitats.

Materials and methods

Field collections and morphometric measurements

Argia sedula, Coenagrionidae, a common damselfly in the USA, ranges from Florida north to Pennsylvania, west to California, and south into Mexico. This wide distribution covers the southern two-thirds of the USA (Abbott, 2005). Populations inhabit rivers with gentle current, and streams as well as ponds, lakes, and wetlands. Males have a predominantly black abdomen with bright blue rings while the females are a light brown (Paulson, 2011). Their reproductive life averages four to five days with a maximum of two weeks (Westfall & May, 2006). Our extensive collections of *A. sedula* from 2009–2015 included 977 males and 449 females from five locations and provided a database with seasonal, habitat, and annual dimensions (Table 1). Various subsets of this database were selected to test our hypotheses. Selections of these subsets were primarily based on adequate sample sizes (n $\geq$ 30) for the hypotheses being tested.

To examine variations among wing shapes and wing sizes of adults developing and emerging from aquatic locations and environments, collections from four sites in Bell, Falls, and McLennan counties, Texas, USA were used. Falls on the Brazos (FOB), a site on the Brazos River (Falls County, 31.25 N, 96.92 W), has a rocky bottom and is approximately 150 m wide. Shoreline depth is 0.5 m, and velocity is 5 m s^{-1}. Nolan Creek (Bell County, 31.04 N, 97.44 W) is approximately 25 m wide, shoreline depth 0.3 m, and velocity 2 m s^{-1}. Harris Creek (McLennan County, 31.46 N, 97.29 W) has a width of approximately 8 m, a shoreline depth of 0.25 m, and intermittent flow of 0-1 m s^{-1}. The Lake Waco Wetlands (McLennan County, 31.61 N, 97.30 W) covers 75 hectares with a residence time of eight days. Water flows through the Wetlands from the Bosque River into Lake Waco.

To examine annual variations among wing shapes and wing sizes of adults collected from the same environment but different years, data subsets were used for each year from 2009–2011, and 2014–2015 collections.

Table 1. Localities, years, and sample sizes collected for females and males of the damselfly *Argia sedula*.

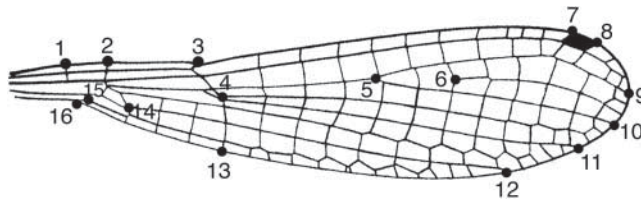|	Year	Females (n)	Males (n)
Falls on the Brazos (FOB)	2009	40	60
	2014	Total = 132	Total = 283
	2014	Early = 34	Early = 79
	2014	Late = 30	Late = 72
Lake Waco Wetlands	2009	Total = 136	Total = 257
	2009	Early = 36	Early = 64
	2009	Late = 62	Late = 124
	2010	118	130
	2011	56	114
Harris Creek	2015	0	49
Nolan Creek	2015	0	44
Totals		482	937

Figure 1.   Right forewing of *Argia sedula* with landmark positions defining wing shape. All landmarks were consistent for both sexes.

To examine variations in wing shape and wing sizes of adults emerging early versus late in their flight season, data subsets from 2009 and 2014 and from two contrasting habitats were used. Early flight season (May–June) included adults whose larval development was primarily over winter. Late flight season (August–October) included adults whose larvae developed primarily in spring and summer temperatures.

Damselflies were netted, transported to the laboratory, killed using ethyl acetate, and weighed. Wings were spread and scanned at 800 dpi. Damaged wings were excluded from our analyses. To obtain shape data, 16 homologous landmarks were designated on scans of the right forewings and digitized using tps.Dig2 software (Rohlf, 2015a) (Figure 1). Landmarks were either at vein intersections or where veins reached the wing edge. Reliable and consistent landmarks were located on all wing images (McTavish, Smith, Guerrero, & Gering, 2012). Wing size was quantified as centroid size, which is the square root of the sum of the squared distances of the set of landmarks from their centroid (Outomuro et al., 2012). Wing size commonly and reliably estimates body size (e.g. abdomen length) (Outomuro & Johansson, 2011; Sadeghi, Adriaens, & Dumont, 2009). To evaluate measurement error, repeat measurements were taken for a subset of wings, and a Procrustes ANOVA was performed using MorphoJ software (Klingenberg, 2011). Results indicated that measurement error was not significant.

Data analysis

Wing shape variations were analyzed using geometric morphometrics which rotates, translates, and scales landmark configurations into alignment via generalized least squares Procrustes superimposition (Klingenberg & McIntyre, 1998). Procrustes fit removes variation in scale, position, and orientation, but preserves shape (Outomuro & Johansson, 2011). To remove the allometric component of wing shape, a multivariate linear regression of the coordinates of the aligned configurations on centroid size was performed using MorphoJ (Klingenberg, 2011). Further analyses employed residuals from the regressions as the size-corrected shape variables (Klingenberg & McIntyre, 1998; Outomuro et al, 2013). Analyses were performed only on right forewings.

Given the unknown distribution of Procrustes coordinate data, we used permutation multivariate analysis of variance (PERMANOVA) with 10,000 random samples on size-corrected shape variables to assess F-ratio significance for variance differences (Hoffmann et al., 2002; Slice, 2007). *P*-values were calculated from Wilks' lambda statistics. Comparisons of shape data were conducted using PAST software (Hammer, Harper, & Ryan, 2007) to assess whether differences in wing shape were associated with sampling location, seasonality, or annual variation (McTavish et al., 2012).

Principal components reduce data by summarizing multidimensional variation effectively in fewer dimensions (Klingenberg & McIntyre, 1998). For meaningful analyses, the first few principal components (PCs) should account for most of the total variation contained in a dataset. PCA of the size-corrected shape variables was conducted with MorphoJ (Klingenberg, 2011), which displayed patterns of variation as scatterplots and identified landmarks with the highest

loading coefficients for positions most likely responsible for shape differences (Klingenberg & McIntyre, 1998). To visualize wing shape variations between groups, tps.Splin software (Rohlf, 2004) was used to create deformation grids comparing the mean shape of the group with the mean for the merged groups (Outomuro & Johansson, 2011).

Mean centroid size of wings was calculated for each population to assess variation in overall wing size, and therefore overall body size. Correlation between wing centroid size and body size is quite high (Outomuro & Johansson, 2011). Centroid sizes were calculated using tpsRelw software (Rohlf, 2015b). A one-way ANOVA conducted with PAST tested significance of centroid size differences among populations, and was followed by Tukey's post hoc test to define pairwise differences (Hammer et al., 2007). Homogeneity of the variance and normality were tested by Levene's test and Shapiro–Wilk test, respectively (Sadeghi & Dumont, 2014).

Results

Early versus late flight seasons

Wing shape

Significant differences in wing shape were consistently found in all comparisons made between early emerging populations and late emerging populations. Wing shape varied significantly for early versus late flight seasons for compared populations in two years (2009, 2014), in two environments (Wetlands, FOB), and for both sexes. Other years and locations in our database had inadequate sample sizes (n < 30) for testing between early and late seasons. PERMANOVA on the size-corrected shape variables indicated that wing shapes of females from Wetlands 2009 (F = 2.66, $p = 0.03$) and FOB 2014 (F = 9.17, $p < 0.001$) varied significantly between early versus late seasons. Similarly, for males, wing shapes of adults from Wetlands 2009 (F = 4.84, $p < 0.001$) and FOB 2014 (F = 17.52, $p < 0.001$) varied significantly between early versus late flight seasons.

PCA on size-corrected shape variables indicated that the first two PCs accounted for 69.5% (females, 2009), 56% (males, 2009), 62.5% (females, 2014), and 54% (males, 2014) of the total variation in each comparison. Scatterplots visualized variation along the first two PCs for the comparisons between early and late season wing shapes (Figure 2). Deformation grids visualized the mean wing shape for each group (Figure 3).

Centroid size

Centroid sizes as measures of overall wing sizes differed significantly for adults from early versus late flight seasons for populations compared from two years (2009, 2014), in two environments (Wetlands, FOB), and both sexes. For both males and females, wings of early emergers were larger than wings of late emergers. ANOVA indicated that wings of females sampled early season (centroid $\bar{x}$ = 893.27) were significantly larger than wings of females sampled late season (centroid $\bar{x}$ = 854.42) from Wetlands 2009 (F = 12.28, $p < 0.001$). Wings of females sampled early season (centroid $\bar{x}$ = 944.72) were significantly larger than wings of females sampled late season (centroid $\bar{x}$ = 848.99) from FOB 2014 (F = 13.61, $p < 0.001$) (Figure 4).

Wings of males sampled early season (centroid $\bar{x}$ = 832.98) were significantly larger than wings of males sampled late season (centroid $\bar{x}$ = 805.03) from Wetlands 2009 (F = 27.20, $p < 0.001$). Wings of males sampled early season (centroid $\bar{x}$ = 909.48) were significantly larger than wings of males sampled late season (centroid $\bar{x}$ = 801.55) from FOB 2014 (F = 76.27, $p < 0.001$) (Figure 4).
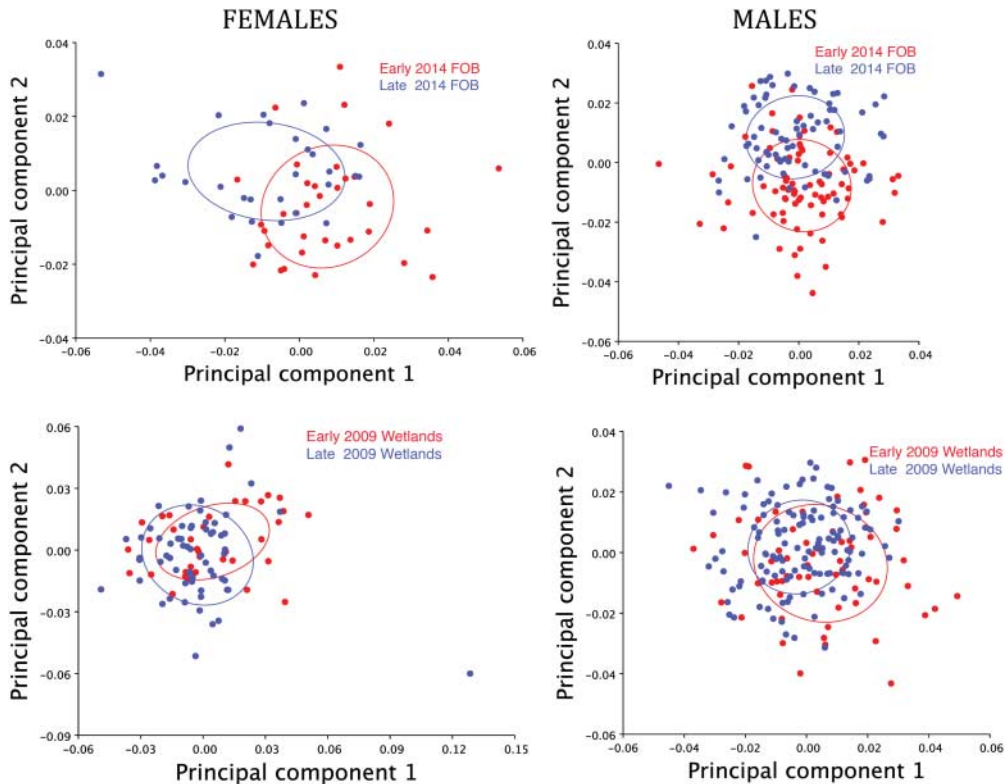
Figure 2. Scatterplots from PCA comparing wing shapes of females and of males collected early in the flight season with those collected late in the flight season in Wetlands 2009 and FOB 2014. Ellipses represent 50% frequency.

Variation among habitat types

Wing shape

Variation in wing shape was between female populations sampled from two locations (Wetlands, FOB), and between male populations sampled from four locations (Wetlands, FOB and Harris Creek, Nolan Creek). No significant differences were found between any locations sampled for both males and females. PERMANOVA on the size-corrected shape variables indicated that wing shapes of females from Wetlands 2009 did not differ significantly from wing shapes of females from FOB 2009 (F = 2.27, p = 0.057). Wing shapes of males sampled at Wetlands 2009 did not differ significantly from those sampled at FOB 2009 (F = 1.19, p = 0.29). Likewise, wing shapes of males sampled at Harris Creek 2015 did not differ significantly from those of males sampled at the similar stream habitat Nolan Creek 2015 (F = 0.70, p = 0.64).

The first two components of the PC analysis of size-corrected shape variables accounted for 57% of the total variation in each of the comparisons between locations. Variation along the first two PCs was shown in scatterplots for each comparison of locations (Figure 5). Deformation grids visualized the mean wing shape for each group (Figure 6).

Centroid size

Differences in wing centroid sizes were compared for females sampled from two locations (Wetlands, FOB) and for males sampled from four locations (Wetlands, FOB, and Harris Creek,
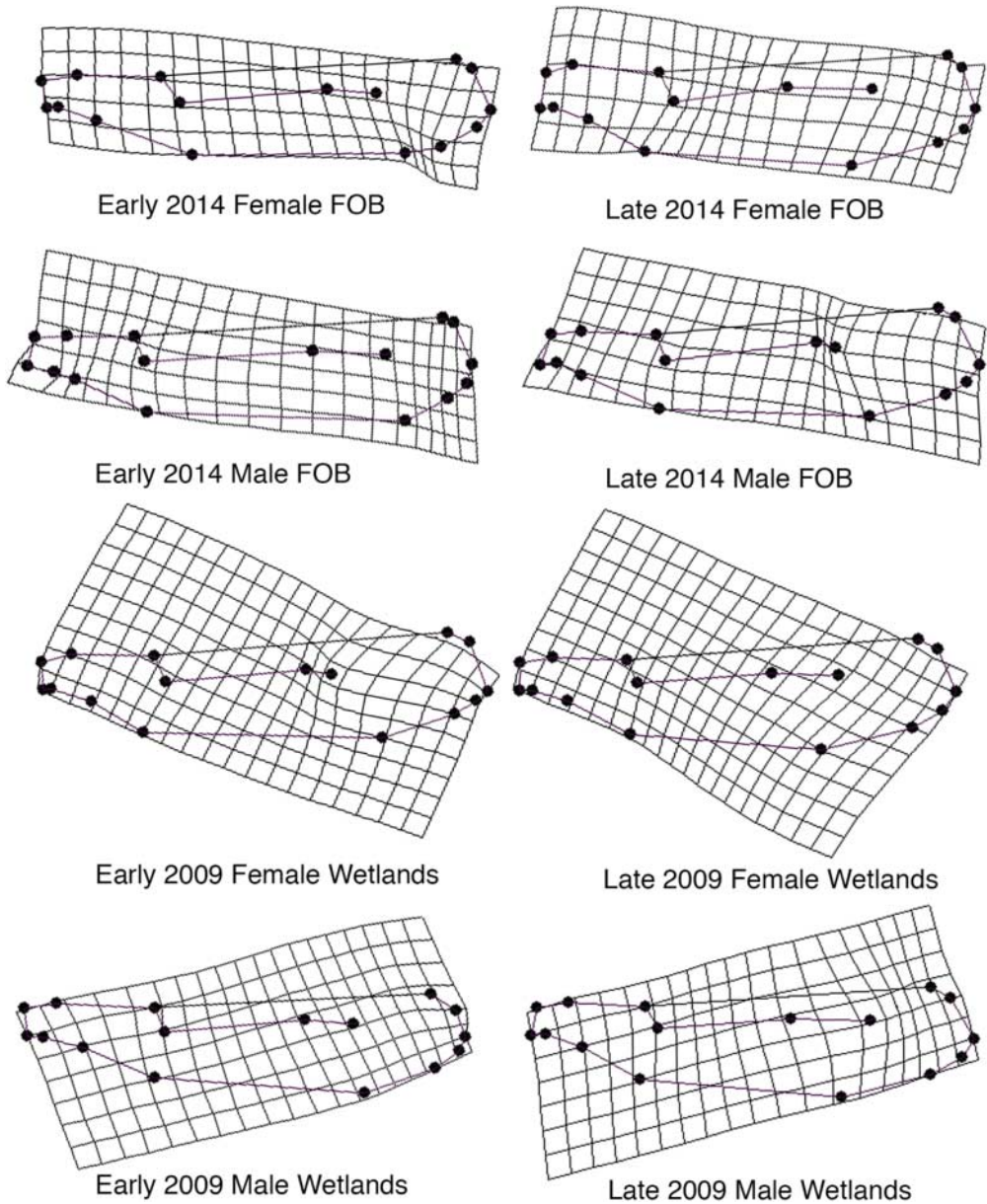
Figure 3.   Variation of wing shape between females and males collected early versus those collected late in the flight seasons of Wetlands 2009 and FOB 2014. The deformation grids indicate the mean shape of each group in relation to the overall mean of the groups being compared. Minimal increases in factor level were occasionally used to highlight differences.

Nolan Creek). Significant differences were found only in the male populations collected in 2009, with males sampled from the Wetlands larger than those sampled at FOB. ANOVA indicated that wing centroid sizes of females from Wetlands 2009 (centroid $\bar{x} = 878.28$) were not significantly different from those from FOB 2009 (centroid $\bar{x} = 869.66$) (F = 0.666, $p = 0.416$) (Figure 7). Centroid sizes of males from Wetlands 2009 (centroid $\bar{x} = 826.52$) were significantly larger than those of males from FOB 2009 (centroid $\bar{x} = 802.88$) (F = 15.12, $p < 0.001$) (Figure 7).
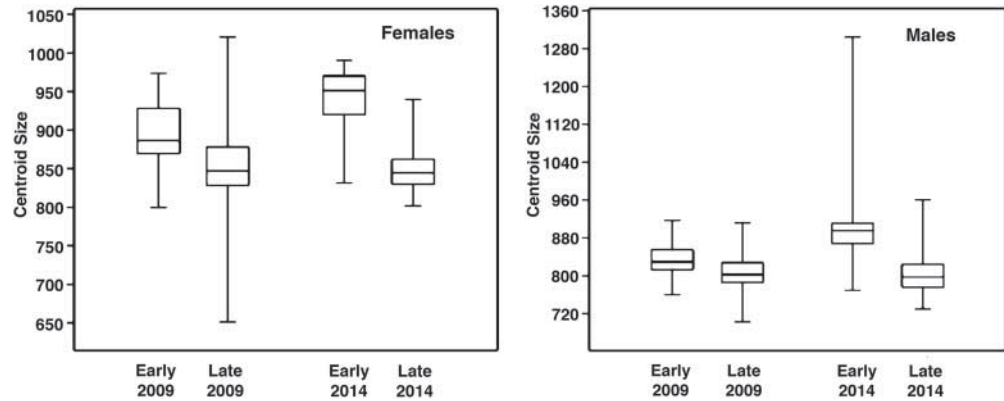
Figure 4.   Mean centroid sizes of females and males collected early and those collected late in the flight seasons of Wetlands 2009 and FOB 2014. Note difference in y-axes.
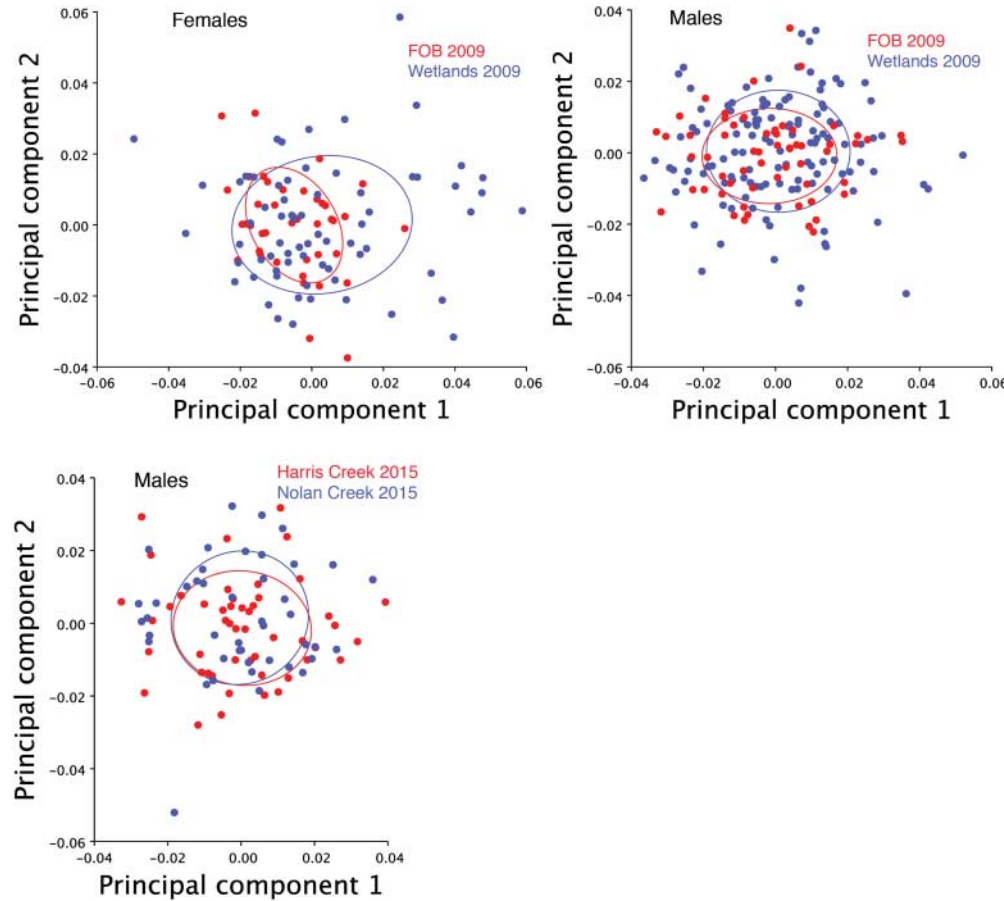

Figure 5.   Scatterplots from PCA comparing wing shapes of females and males collected at Wetlands 2009 and FOB 2009; and males collected in Harris Creek 2015 and Nolan Creek 2015. Ellipses represent 50% frequency.

Wing centroid sizes of males from Harris Creek 2015 (centroid $\bar{x} = 831.00$) were not significantly different from those of males from Nolan Creek 2015 (centroid $\bar{x} = 838.31$) (F = 1.19, $p = 0.278$).
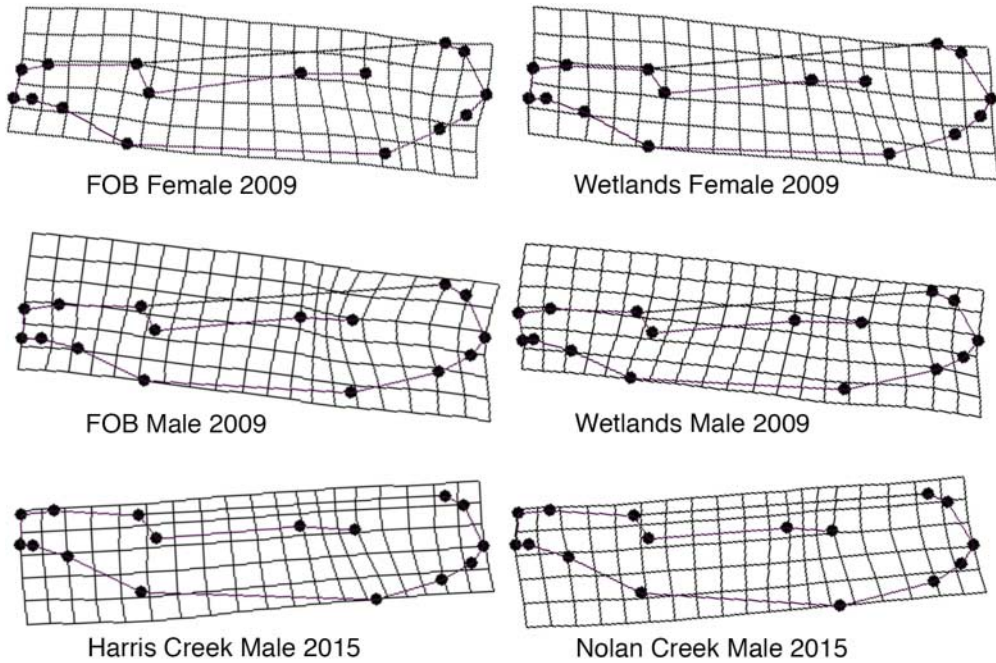
Figure 6. Variation of wing shape between females and males collected at Wetlands 2009 and FOB 2009; and males collected at Harris Creek 2015 and Nolan Creek 2015. The deformation grids indicate the mean shape of each group in relation to the overall mean of the groups being compared. Minimal increases in factor level were occasionally used to highlight differences.
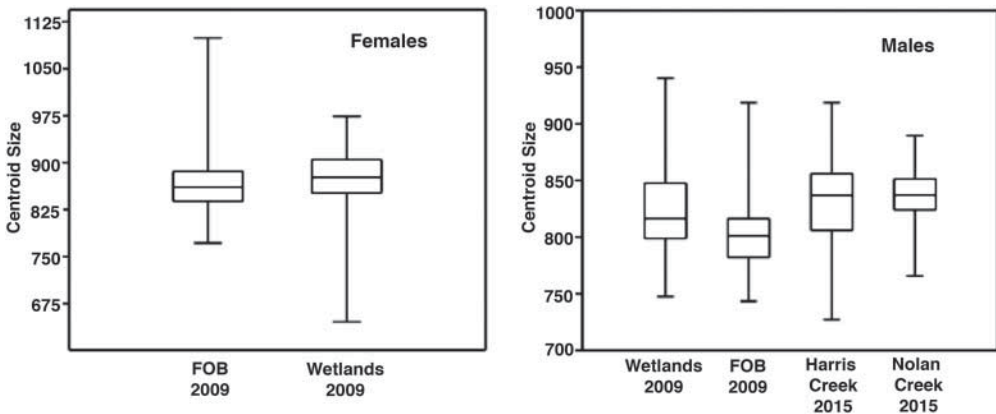

Figure 7. Mean centroid sizes of females and males collected in Wetlands 2009 and FOB 2009; and of males collected at Harris Creek 2015 and Nolan Creek 2015. Note difference in y-axes.

Annual variation

Wing shape

Variation in wing shape for females and males was examined for collections from 2009 and 2014 at FOB and from 2009, 2010, and 2011 at the Wetlands. Significant differences in shape were found in comparisons between male populations and between female populations sampled from
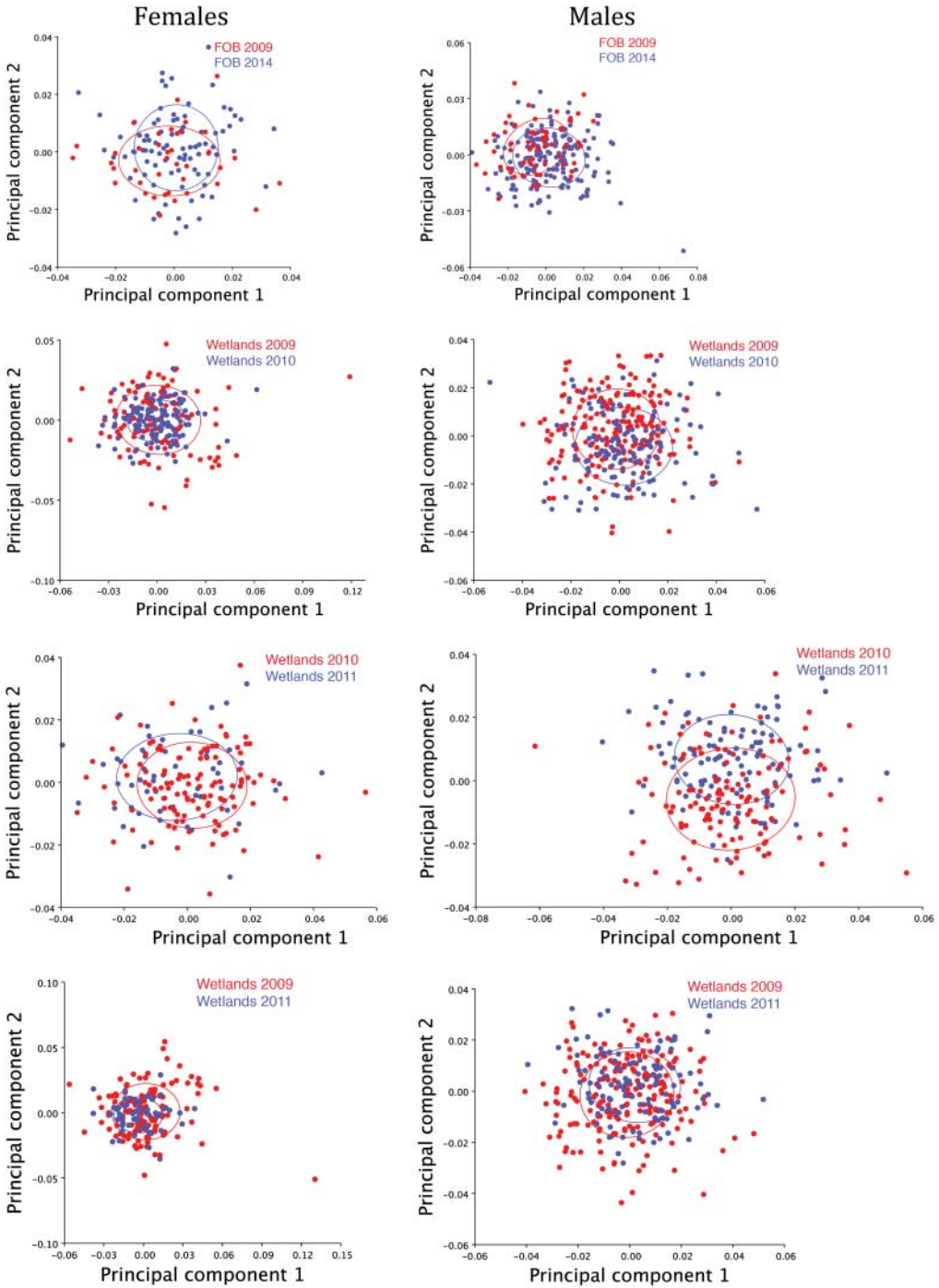
Figure 8.   Scatterplots from PCA comparing wing shapes of females and males collected at Wetlands 2009, 2010 and 2011; and FOB 2009, 2014. Ellipses represent 50% frequency.

FOB and from the Wetlands. For samples from FOB 2009 versus FOB 2014, PERMANOVA on the size-corrected shape variables revealed significant differences between wing shapes for females (F = 2.76, $p = 0.02$) and for males (F = 5.83, $p < 0.001$).
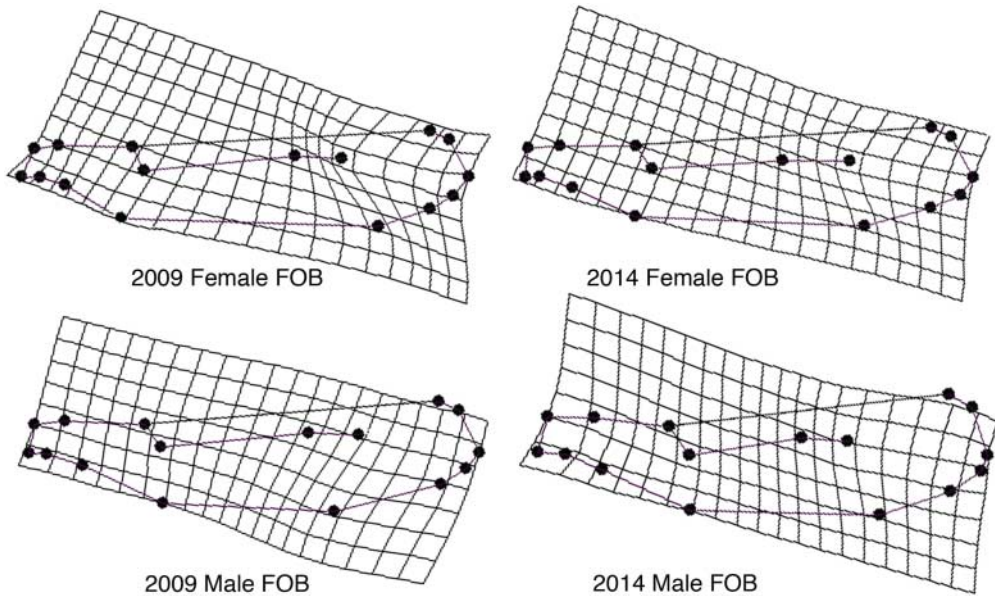
Figure 9.   Variation of wing shapes between females and males collected at FOB 2009 and 2014. The deformation grids indicate the mean shape of each group in relation to the overall mean of the groups being compared. Minimal increases in factor level were occasionally used to highlight differences.

Females and males were also sampled from Wetlands in consecutive years 2009, 2010, and 2011. Mean wing shapes for females were significantly different among years (PERMANOVA, $F = 4.33$, $p < 0.001$), as were wing shapes for males (PERMANOVA, $F = 8.36$, $p < 0.001$). In pairwise comparisons between years, female wing shapes were significantly different between 2009, 2010 ($p < 0.001$) and 2010, 2011 ($p < 0.001$), but not significantly different between 2009, 2011 ($p = 0.09$). Wing shapes of males showed a similar pattern with significant differences between years 2009, 2010 ($p < 0.01$) and 2010, 2011 ($p < 0.01$), and no significant differences between years 2009, 2011 ($p = 0.08$).

PCA on size-corrected shape variables showed that the first two PCs accounted for 54% (females, 2009 and 2014), and 56% (males, 2009 and 2014) of the total variation in wing shape. The first two PCs accounted for 60% (females, 2009 and 2010), 51% (females, 2010 and 2011), 66% (females, 2009 and 2011) and 57.5% (males, 2009 and 2010), 58% (males, 2010 and 2011), 54% (males, 2009 and 2011) of the total variation in wing shape. Scatterplots illustrate variation along PC1 and PC2 (Figure 8). Deformation grids visualize the mean wing shape for each group (Figures 9, 10).

Centroid size

Annual differences in wing centroid sizes for females and for males were examined for collections from four years (2009, 2010, 2011, 2014). Significant size differences were found between male populations collected at FOB and between those collected at the Wetlands. Wing sizes for the female comparison from FOB were significantly different, as were two of the three female comparisons made at the Wetlands. ANOVA indicated that wing centroid sizes of females from FOB 2009 (centroid $\bar{x} = 869.66$) were significantly smaller than those for females from FOB 2014 (centroid $\bar{x} = 904.32$) ($F = 12.61$, $p < 0.001$). Wing centroid sizes for males from FOB 2009 (centroid $\bar{x} = 802.88$) were significantly smaller than those of males from FOB 2014 (centroid $\bar{x} = 868.51$) ($F = 38.70$, $p < 0.001$).
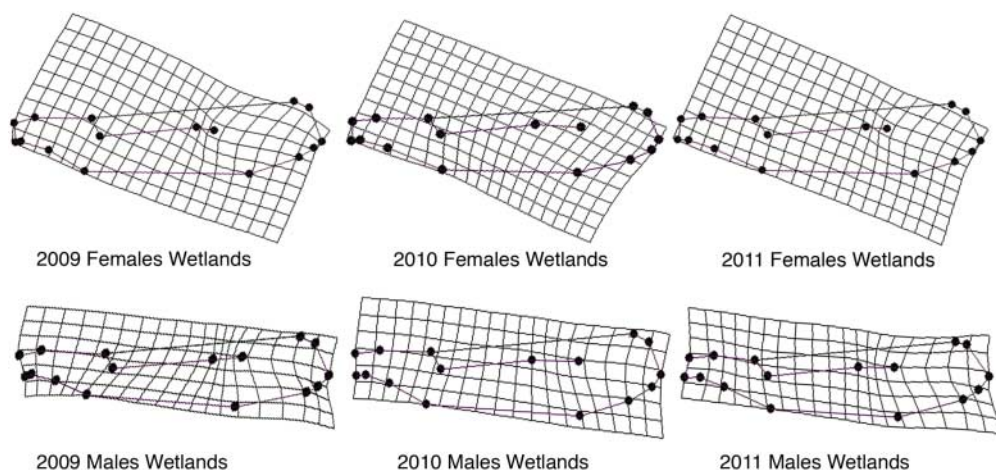
Figure 10. Variation of wing shapes between females and males collected at Wetlands 2009, 2010, and 2011. The deformation grids indicate the mean shape of each group in relation to the overall mean of the groups being compared. Minimal increases in factor level were occasionally used to highlight differences.
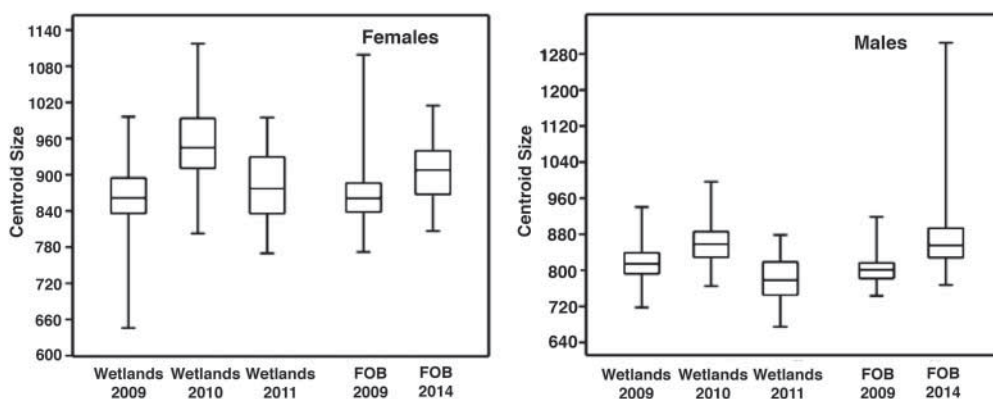

Figure 11. Mean centroid sizes of females and males collected at Wetlands 2009, 2010, and 2011 and at FOB 2009, 2014. Note difference in y-axes.

ANOVA indicated significant differences among years for centroid sizes of females from Wetlands 2009, 2010 and 2011 (F = 77.42, $p < 0.001$). Pair-wise comparisons indicated significant differences in centroid sizes for females from 2009 versus 2010 (Tukey's Q = 14.35, $p < 0.001$) and from 2010 versus 2011 (Tukey's Q = 12.03, $p < 0.001$). However, centroid sizes for females from 2009 versus 2011 were not significantly different (Tukey's Q = 2.32, $p = 0.228$). For these female populations, wing sizes were largest in 2010 (centroid $\bar{x} = 951.97$), compared to 2011 (centroid $\bar{x} = 880.53$) and 2009 (centroid $\bar{x} = 866.74$) (Figure 11).

ANOVA indicated significant differences among years for centroid sizes for males from Wetlands 2009, 2010 and 2011 (F = 105.20, $p < 0.001$). Pair-wise comparisons also indicated significant differences for males from 2009 versus 2010 (Tukey's Q = 10.87, $p < 0.001$), 2009 versus 2011 (Tukey's Q = 11.21, $p < 0.001$), and 2010 versus 2011 (Tukey's Q = 22.08, $p < 0.001$). For these male populations wing sizes were largest in 2010 (centroid $\bar{x} = 855.88$) compared to wing sizes in 2009 (centroid $\bar{x} = 818.10$) and in 2011 (centroid $\bar{x} = 779.12$) (Figure 11).

Discussion

Environmental conditions affect morphology of organisms (Outomuro & Johansson, 2011), as genetically similar organisms developing in different conditions may display quite different characteristics (Stearns, 1989). The morphology of insect wings can largely determine the energetic costs and maneuverability of flight (Betts & Wootton, 1988). This study examined the effects of environmental conditions on wing shape and wing size in the damselfly *A. sedula.* We examined these morphologies of males and females emerging early versus late in the flight season, between damselfly populations from different locations in the same year, and among damselflies emerging from the same location but in different years. Wing shape and wing size varied significantly in most comparisons, suggesting that environmental factors consistently influence wing shape and body size. The frequency of wing shape and wing size differences supports the concept that whole body morphology is a thorough integration of all components of size and shape.

Comparisons of early versus late emerging damselflies showed consistent differences for both wing shape and wing size for all comparisons. Wing shape and wing size, as components of flight morphology, have shown seasonal variation in other studies (Bots et al., 2009; Debat, Bégin, Legrout, & David, 2003; Klingenberg, 2013; Pétavy et al., 1997; Norberg & Leimar, 2002). In our study, examination of deformation grids revealed that both male and female populations collected at the Wetlands in 2009 and at FOB in 2014 had wider wings late in the season, which would aid maneuverability (Betts & Wootton, 1988). Internal landmark (6) was also frequently variable, and likely contributes to wing stability (Bots et al., 2009).

Wing centroid sizes, and therefore body sizes, were larger for adults collected in early flight season for all years, locations, and both sexes. Other studies have noted similar results for wing size variation (Bots et al., 2009; Norberg & Leimar, 2002). Insects emerging early in the flight season and therefore developing in colder temperatures are frequently larger than those emerging late in the season after developing in warmer temperatures (Bots et al., 2009; Norberg & Leimar, 2002).

Unexpectedly, wing shape for females and for males was not sensitive to environmental differences between the Wetlands and the riverine FOB. Wing sizes also were not significantly different between female populations. However, wing size was larger for males developing at the Wetlands. Interestingly, the body sizes of the most common species of the damselfly community at the Wetlands are smaller (e.g. *Enallagma* spp., *Ischnura* spp.) than those dominant species at the FOB community (*Hetaerina* spp., *Argia* spp.). This difference could be influenced by interspecific interactions. Life histories differ for the territorial *Argia sedula*, which results in different flight patterns for males and females. The observed differences in wing sizes between males but not females might be explained by male territorial behavior. Water discharge and velocity vary greatly between these two locations, with greater discharge and velocity at the riverine FOB. These differences may have contributed to significant wing size variation in males at the contrasting environments. As expected, wing shape and wing size of males collected from Nolan Creek and Harris Creek also did not differ significantly for these similar stream habitats.

Annual differences in wing shape occurred for most populations compared between two years. Norberg and Leimar (2002) found that annual variations in environmental influences on insect morphology were considerable. Damselflies were collected for three years at the Wetlands (2009–2011) and for two years at FOB (2009, 2014). All male and female populations from both locations showed considerable annual variation, especially in landmark (12) on the lower edge of the wing affecting wing width. Variation in all populations was also notable for internal landmark (6) which likely influences wing stability. In males and females, wing sizes differed significantly among Wetland collections from all three years with largest wings in 2010. At FOB, wing sizes of both males and females varied significantly, with larger wings occurring in 2014. Water levels at the Wetlands varied considerably over these three years with the draining of the

Wetlands in July 2010 and drought conditions in 2011. The water regime of the area they inhabit is an environmental factor of primary importance. Their abundance can depend directly on water level and decrease as this level drops (Popova, Haritonov, & Erdakov, 2016).

Wing shape evolution and adaptation involves environmental pressures that optimize efficiency (Outomuro et al., 2013). Our research indicates that significant morphological variation resides in the sensitivity of wing shape development to annual and seasonal variation. Habitat influences on wing shape and body size were not as pronounced as were annual and seasonal differences. The concept that insects embody a rigid, purely deterministic shape should not be assumed. Significant plasticity expressed during development occurs in the damselfly genome. Further research should document the precise relationships between habitat parameters and measurable morphological features.

Acknowledgements

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